

In Vivo Research of Time-Lapse Changes on Surgical Sutures by OCT Signal Analysis

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Abstract

Currently, animal and clinical research on biomaterials, such as surgical sutures, are mainly performed by removing them from the experiment targets and observing them by microscopy. However, traditional microscopy is not able to observe the internal structure, and there is a risk of sacrificing animals to remove the suture and damaging the materials. Therefore, we introduced optical coherence tomography (OCT) to observe and evaluate four different kinds of surgical sutures in vivo (monofilament absorbable and nonabsorbable sutures and braided absorbable and nonabsorbable sutures). As a result, while the monofilament nonabsorbable sutures showed almost no change over time, the absorbable sutures had color fading and it was also confirmed that the internal structure became chaotic due to decomposition, which improved the OCT signal intensity. For the braided sutures, both absorbable and nonabsorbable, we found that the reflection signal improved from week 0 because blood got among the filaments of sutures and dried during recovery which increased OCT signal from week 0 to week 1. We also confirmed that the braided sutures untwisted over time. All four kinds of sutures were pulled due to the movement of rats during recovery. It is expected that OCT technology will be of great help in *in vivo* experiments on biomaterials such as sutures.

Keywords

Optical Coherence Tomography, Surgical Suture, Biomedical, PLA/PCL, PVDF, PGA, PEs, *In Vivo* Animal Experiment

1. Introduction

Since optical coherence tomography (OCT) technology was developed in the 1990s [1] with the advantages of high speed, high resolution, and non-invasiveness, it has

been widely used in bio-measurement and industrial applications. Especially in the field of medical biomedical measurements, OCT measurements have been able to distinguish between mild cognitive impairment and Alzheimer's disease by scanning eye tissues [2]. Using OCT angiography, researchers also successfully assessed ocular vascular density in humans across age groups [3]. OCT technology is playing an important role in ophthalmology and ocular tissue measurement. Furthermore, OCT devices are not only useful in ophthalmology, but also using a light source with a central wavelength of 1300 nm are particularly attractive for measuring skin tissue and for the development of endoscopic OCT devices [4]. For example, Ohmi *et al.* succeed to measure and analyze soft tissues, such as muscle, and hard tissues, such as bone and teeth by OCT technology [5].

In our laboratory, we are also conducting research to visualize and quantitatively analyze the sweating behavior of the sweat glands underneath the fingertips when humans experience emotional sweating [6]. Furthermore, animal experiments using guinea pigs were conducted to investigate allergic reactions that appear on the skin [7]. In addition, animal experiments were performed to evaluate the change in diameter of surgical sutures due to water absorption, deterioration, and decomposition [8]. However, surgical sutures are not limited to absorbable and non-absorbable monofilament; there are many other types of sutures available. Examples include spider silk-infused braided yarns [9] and surgical braided nanofibers based on electrospinning technology [10]. While surgical sutures are widely used in clinical practice, there have been few studies on the in vivo observation and analysis of the internal structure of fibers. In addition, materials used in absorbable sutures, such as polylactic acid, may show differences in signal intensity and absorption peaks due to differences and changes in the internal structure in experiments investigating infrared spectra such as FTIR [11]. The ability to analyze the internal structure of surgical sutures in vivo will enable a better understanding of the mechanisms of suture decomposition and degradation, which could be useful in improving the biocompatibility of sutures and controlling their mechanical performance.

Based on the above previous studies, we hypothesized that not only the diameter of surgical sutures but also changes in the internal structures which affect OCT signal intensity can be measured by OCT, and we conducted research by adding one more kind of suture, which is braided sutures, with both absorbable and nonabsorbable. And we sutured surgical sutures to the backs of hairless rats, performed *in vivo* OCT observations, and attempted to evaluate the changes in surgical sutures over time by analyzing the obtained OCT images and signal intensity.

2. Methods

2.1. Suture Surgery

Two 10-week-old male hairless rats (SPF, HWY/slc, body weight 280 g) were used and kept in a constant temperature environment. They were allowed to have free access to solid food and tap water. The animal experiments were conducted under the permission of the Animal Experiment Ethics Committee of the Graduate School of Medicine, Osaka University. The sutures used were 6-0 (approximately 70 - 100 μ m) monofilament polyvinylidene fluoride (PVDF) non-absorbable suture (AR526, Kono Seisakusho, blue) and polylactic acid/polycaprolactone (PLA/CL) composite monofilament absorbable suture (LC516, Kono Seisakusho, purple) for one hairless rat, and 6-0 braided polyester (PEs) non-absorbable suture (TP726D, Kono Seisakusho, green) and braided polyglycolic acid (PGA) absorbable suture (VB116, Kono Seisakusho, white) for the remaining hairless rat.

For the suturing surgery, anesthesia was administered by intraperitoneal administration of a three-type mixed anesthetic. The amount of anesthetic used is shown in **Table 1**. After the animal's pain reflex disappeared, the suturing surgery began. Four parallel incisions were made on the back of the animal, each about 2 cm long, and four simple sutures were placed at intervals of about 4 mm. The entire suturing surgery took about 30 minutes.

Table 1. Injection volume of drugs used in anesthesia.

	X axial	Z axial
Size/pixel	648	415
FOV/mm	2.25	1.44
Resolution/ μ m·pixel ⁻¹	3.47	3.47

2.2. OCT Tomography

The experiment was conducted using a Spectral Domain OCT device (Telesto 320, Thorlab, central wavelength 1300 nm, maximum imaging depth: air 3.6 μ m/water 2.6 μ m, axial resolution: air 5.5 μ m/water 4.2 μ m). 2D-OCT images were taken once on the day of the suture surgery and once a week thereafter, until the absorbable suture broke. Approximately six images were taken of each of the 16 sutured locations. The imaging parameters for the OCT images are shown in Table 2.

Table 2. Parameter settings of OCT.

Drug	Injection Volume/mg/ml/kg	
Medetomidine	0.15 mg/0.15ml/Kg	
Midazolam	2 mg/0.4ml/Kg	
Butorphanol Tartrate	2.5 mg/0.5ml/Kg	
Saline Solution	1.45 ml/Kg	

3. Results

3.1. OCT Tomographic of Surgical Sutures

The OCT images and digital camera images obtained are shown in **Figure 1** and **Figure 2**. The monofilament absorbable suture was cut on the 9th week due to

complete decomposition, as a result OCT images could be taken up to the 8th week. However, because the absorbable braided suture was cut during the 4th week, images could only be taken up to the 3rd week.



Figure 1. OCT tomographs and digital camera pictures of monofilament. Absorbable suture is shown on the left and non-absorbable suture is shown on the right. Suture is indicated by red arrows, and we can see absorbable suture is almost transparent while non-absorbable suture reflected lots of signals. Green arrows indicate the attachment around suture which is mainly consisted by blood. Yellow arrows show the distance between suture and stratum corneum. We found that suture would separate from skin and got loosen due to the movement of rats.



Figure 2. OCT tomographs and digital camera pictures of braided suture. Absorbable suture is shown on the left and non-absorbable suture is shown on the right. Suture is indicated by red arrows. Because of the space exist among the filament of braided suture, blood infiltrate into the suture. And the reflection from the surface of filament also can be confirmed.

Figure 1 shows images of two types of monofilament sutures at 0, 4, and 8 weeks. The left side is absorbable sutures, and the right side is non-absorbable suture. Digital camera images of absorbable sutures show that absorbable sutures have faded from

their original purple color to transparent. In contrast, non-absorbable sutures have remained blue, and no change has been observed. This proves that the absorbable sutures are decomposing. This is consistent with the results of our previous study [8]. The absorbable and non-absorbable sutures are both indicated by red arrows in the OCT images. The absorbable sutures are made of PLA/CL material and absorb 1300 nm light so much that the OCT signal is weak and appears in black. The non-absorbable sutures are made of PVDF and reflect a lot of 1300 nm light and appear in white. This suggests that OCT technology can be used to distinguish the types of sutures to a certain extent as well. The surrounding tissue is indicated by green arrows. Looking at the condition of the non-absorbable sutures over 4 - 8 weeks, it was found that the amount of adhesions around the fibers increased compared to 0 - 4 weeks, and then by the 4th week the wound had almost healed, causing the bleeding to almost completely disappear and decreased the OCT signal intensity over the 4 - 8th week. It is assumed that the adhesions around the thread are mainly scabs formed by dried blood. Furthermore, the distance from the skin surface to the suture itself is indicated by two yellow arrows. Immediately after surgery (0 weeks), both the absorbable and non-absorbable threads were firmly sutured to the skin, but as time passes, they become looser, and in the 2D-OCT images they were separated from the skin surface. In digital camera images, the sutures appear to be floating above the skin, along with both types. This is thought to be because the rats were moving during the post-operative recovery process, causing the firmly sutured sutures to be pulled.

Figure 2 shows images of absorbable and nonabsorbable braided sutures at 0 -3 weeks. The left side shows absorbable sutures, and the right side shows nonabsorbable sutures. In the digital camera image at 0 weeks, blood has gone into the space among the filaments of the suture fibers because the suture is braided. It is suspected that braided sutures are more likely to cause inflammatory and allergic reactions than monofilaments because of the blood left in the voids among those filaments. In addition, when comparing the digital camera image at 3 weeks with 0 weeks, it was confirmed that both absorbable and nonabsorbable sutures had untwisted themselves. In other words, regardless of absorbency, braided sutures could untwist over time, suggesting the risk of their mechanical properties decreasing could be faster than monofilaments. It is speculated that the mechanical properties of absorbable braided sutures may further deteriorate as the fibers decompose. This is also thought to be one of the reasons why the absorbable braided suture could not withstand 4 weeks and broke. The sutures are indicated by a red arrow in the OCT image, just like the monofilament. Both braided sutures reflected a lot of light, so it appears as strong signals. Furthermore, it was found that the dimension of both absorbable and nonabsorbable sutures increased with time lapse. This proved that the sutures were untwisted as mentioned above. In addition, a slightly stronger signal was observed on the surface of the filaments that make up the braided sutures. This is thought to be an interface reflection.

3.2. Analysis of OCT Signal Intensity

The analysis of the OCT signal intensity of the suture body is shown in **Figure 3**. **Figure 3(a)** shows the data for monofilament, and **Figure 3(b)** shows the data for braided sutures. For monofilament, the non-absorbable sutures (blue triangle) were not absorbed and showed almost no change, but the absorbable sutures (purple circle) showed a significant increase in intensity from week 0 to week 8 (p > 0.03). As speculated in a previous study [8], this is thought to be due to the decomposition of the uniform crystals inside the fiber, which became amorphous, and the disordered structure increased the light reflectance of the sutures. In addition, although the absorbable sutures appear in black in the OCT images with almost no reflected signal, it was found that they have a signal intensity of about 35 to 40 dB.



Figure 3. OCT signal intensity of monofilament (a) and braided suture (b). Non-absorbable monofilament (blue triangle) has almost no changes while absorbable monofilament (purple circle) has a slightly intensity increase from week 0 to week 8 (p > 0.03). Both non-absorbable (green triangle) and absorbable (black circle) braided suture has no changes during week 1 - week 3. However, intensity increased from week 0 to week 1 (p > 0.03) because the water component of blood got into the suture and decrease the whole intensity in week 0.

Focusing on the results for braided sutures, there was no significant difference between absorbable (black circle) and non-absorbable sutures (green triangle) from 1 to 3 weeks. However, there was a significant increase in intensity (p > 0.03) for both absorbable and non-absorbable sutures from 0 to 1 week. It is suggested that blood components with a high-water content entered the voids in the filaments of the braided sutures during surgery, which reduced the reflectance of the entire suture, resulting in the low strength at 0 weeks. One week after surgery, the blood dried and the water content decreased, which was thought to have increased the signal intensity. In addition, the calculation included the strong signal due to the interface reflection mentioned above, so the intensity of the braided sutures (1 to 3 weeks) was about 5 dB higher than the monofilament non-absorbable sutures. The OCT images used to calculate the OCT signal intensity were also used to calculate the signal intensity of the stratum corneum of the skin. The results are shown in **Figure 4**. The images of the two types of monofilament **Figure 4(a)** and the two types of braided **Figure 4(b)** sutures have almost no error. In other words, this study proves that there is no significant difference in the instrumental measurements when calculating the OCT signal intensity.



Figure 4. OCT signal intensity of stratum corneum from OCT tomographs of monofilament (a) and braided suture (b). None of these data has significant difference which means our calculations on suture intensity has no significant error from OCT devices.

4. Conclusions and Discussions

This study demonstrated the reproducibility of previous experiments through OCT images. Monofilament absorbable sutures had a weak signal strength of about 35 - 40 dB, and it was found that the internal structure became disordered as the fibers decomposed, and that their strength also increased. Non-absorbable sutures were strong, at over 50 dB. It was found that OCT could be used to distinguish the types of sutures to a certain extent. In addition, it was confirmed that the sutures were pulled and separated from the skin surface as the rats were active during the postoperative recovery process. It is assumed that the deposits around the threads were mainly dried blood. In addition, blood can penetrate braided threads due to the twisted filaments.

By calculating the signal strength using OCT, it was found that it is possible to observe the decomposition process of sutures and the postoperative recovery process of skin tissue. In the future, it is expected that OCT can be used not only for sutures but also for various polymeric biomaterials to observe the decomposition and deterioration of materials *in vivo*.

What's more, we must admit that 1300nm central wavelength SD-OCT still has limitation on observation depth. We still cannot observe the situation of surgical sutures underneath the skin. So we are considering applying mouse, which has thinner skin to find the changes of sutures among subcutaneous tissues.

Based on this research, according to the successful experiment from the surgical sutures observation on hairless rat, we are planning to increasing the numbers of the experiment targets we used to make sure the data we achieve could get a more reliable result. And we will try to compare OCT data with FTIR and mechanical properties of sutures in the future.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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